Maternal hemoglobin levels and adverse pregnancy outcomes: individual patient data analysis from two prospective UK pregnancy cohorts

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CB, GS, US, GC, AF all declare no conflicts of interest within the scope of this work. Outside the scope of this work: within the last 5 years GS has received research support from Roche Diagnostics Ltd, Illumina and Sera Prognostics (fetal growth restriction, preeclampsia and preterm birth). GS's department has received payment from Roche for a talk given by GS (fetal growth restriction). GS has been a paid consultant to GSK (preterm birth) and is a member of a Data Monitoring Committee for GSK trials of RSV vaccination in pregnancy.

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Short Title

Hemoglobin and pregnancy outcomes

Abbreviations

GDM: Gestational diabetes mellitus

Hb: Hemoglobin

PET: Pre-eclampsia

SGA: Small for gestational age

LBW: Low birth weight

PTB: Preterm birth

ALSPAC: The Avon Longitudinal Study of Parents and Children study

POPS: Pregnancy Outcome Prediction Study

OR: Odds ratio

CI: confidence interval

SD: standard deviation

ABSTRACT

Background

Hemoglobin(Hb) is a modifiable risk factor for adverse pregnancy outcomes. Studies have reported conflicting associations between maternal Hb levels and adverse pregnancy outcomes including preterm birth(PTB), low birth weight(LBW) and perinatal mortality.

Objective

To estimate the shape and magnitude of associations between maternal Hb levels in early(7 to 12 weeks gestation) and late pregnancy(27 to 32 weeks gestation), and pregnancy outcomes in a high-income setting.

Methods

We used data from two UK population-based pregnancy cohorts; The Avon Longitudinal Study of Parents and Children(ALSPAC) and Pregnancy Outcome Prediction Study(POPS). We used multivariable logistic regression models to examine the relationship between Hb and pregnancy outcomes, adjusting for maternal age, ethnicity, BMI, smoking status and parity. Main outcome measures were; PTB, LBW, small for gestational age (SGA), pre-eclampsia(PET), and gestational diabetes mellitus(GDM).

Results

Mean Hb in ALSPAC was 12.5g/dL(SD=0.90) and 11.2 g/dL(SD=0.92) in early and late pregnancy respectively, and 12.7 g/dL(SD=0.82) and 11.4 g/dL(SD=0.82) in POPS.

In the pooled analysis there was no evidence of associations between a higher Hb in early pregnancy(7 to 12 weeks gestation) and PTB(OR per 1 g/dL Hb: 1.09: 95% CI 0.97, 1.22), LBW(1.12:0.99, 1.26) and SGA(1.06;0.97, 1.15). Higher Hb in late pregnancy(27 to 32 weeks gestation) was associated with PTB(1.45:1.30, 1.62), LBW(1.77:1.57, 2.01) and SGA(1.45:1.33, 1.58). Higher Hb in early and late pregnancy was associated with PET in ALSPAC (1.36:1.12, 1.64) and (1.53:1.29, 1.82) respectively, but not in POPS (1.17:0.99,

1.37) and (1.03:0.86, 1.23). There was an association with a higher Hb and GDM in ALSPAC both in early and late pregnancy (1.51:1.08, 2.11) and (1.35:1.01, 1.79) respectively, but not in POPS (0.98:0.81, 1.19) and (0.83: 0.68, 1.02).

Conclusion

Higher maternal Hb may identify risk of adverse pregnancy outcomes. Further research is required to investigate if this association is causal, and to identify underlying mechanisms.

Word Count - Abstract: 313 Article: 3,414 (excluding tables, figures and citations)

Keywords

Hemoglobin, iron-deficiency anemia, low birth weight, preterm birth, pre-eclampsia, small for gestational age, ALSPAC, POPS.

Introduction

Hemoglobin (Hb) is a potentially modifiable risk factor for adverse pregnancy outcomes. Anemia during pregnancy is defined as a Hb concentration lower than 110g/L at sea level. When this is accompanied by an indicator of iron deficiency (hypoferritinaemia) it is referred to as iron deficiency anemia (IDA). Globally 43% of pregnant females are anemic with estimates of nearly 20% in the UK, the vast majority of which is IDA (1). As low Hb associated with IDA is the most common nutritional deficiency worldwide, much attention has focused on preventing IDA. Iron supplementation has become a routine recommendation for women throughout pregnancy with IDA (2). Recent studies however, have raised questions about the value of supplementation in those who are iron-replete and non-anaemic and suggested that excess iron intake may paradoxically increase the risk of adverse pregnancy outcomes (3).

Studies have reported conflicting associations between maternal Hb levels and adverse pregnancy outcomes such as preterm birth (PTB), low birth weight (LBW) and perinatal mortality (3,4). Some studies suggest that low Hb (defined as <11g/L) is associated with increased risks of such adverse pregnancy outcomes, such as LBW, PTB and perinatal death (4, 5), whilst others found that females with higher Hb in pregnancy (≥13 g/L) are at risk of PTB and LBW (6), or have demonstrated a U- shaped associations (7-9). Other evidence has reported that the relationship between Hb levels and PTB or LBW may differ by timing of Hb measurement during pregnancy (7-11). In some studies that examined trimester specific associations these tended to be stronger for lower Hb and adverse pregnancy outcomes in the first trimester, but for higher Hb associations were seen throughout pregnancy, suggesting timing of measurement of maternal Hb levels may be crucial (7-11). In the few studies that directly measured iron status, they demonstrated an association between high iron status and increased risk of PTB and LBW in all three trimesters (9). Challenges however exist in

interpreting iron status without other markers, such of those of inflammation. The concentrations of iron are significantly affected, and fall rapidly as part of the acute phase response after the onset of the inflammation irrespective of the status of the iron stores in the body (3). Further complexity is added by hemodilution, which occurs physiologically in pregnancy. This may result in lower Hb concentrations than in the non-pregnant state (1,3).

The aims of this study are to estimate the shape and magnitude of associations of maternal Hb levels in the first and third trimesters of pregnancy with PTB, LBW (<2500g), small for gestational age (SGA; <10th centile of birth weight for gestational age), pre-eclampsia (PET) and gestational diabetes mellitus (GDM) in a general antenatal population in a high income setting.

Methods

Participants - Two UK Birth cohorts - The Avon Longitudinal Study of Parents and Children (ALSPAC) recruited pregnant females in the former county of Avon, South West England. Females with an expected delivery date between 1 April 1991 and 31 December 1992 were eligible to be included. Full details of the study are found online at http://www.bristol.ac.uk/alspac/researchers//. Ethics approval was granted by the ALSPAC Law and Ethics committee and the local research ethics committee, in compliance with the Declaration of Helsinki http://www.bristol.ac.uk/alspac/researchers/research-ethics/. Detailed antenatal data was abstracted on all 13,706 pregnancies where the medical records could be retrieved. Trained research midwives/nurses abstracted data from medical records. There was no between-midwife variation in mean values of the data abstracted and error rates were consistently <1% in repeated data entry checks. Written informed consent was obtained from both parents and their offspring. For the current study, participants were selected on the basis

of a singleton pregnancy and if they had at least one measure of Hb during pregnancy. Consent for biological samples have been collected in accordance with the Human Tissue Act (2004).

The Pregnancy Outcome Prediction Study (POPS) study is a prospective pregnancy cohort of nulliparous females attending their dating ultrasound scan at the Rosie Hospital (Cambridge, UK) between Jan 14, 2008, and July 31, 2012(12), Those with a viable pregnancy were eligible to participate. The only clinical exclusion criterion was multiple pregnancy. Ethical approval for the study was obtained from the Cambridgeshire 2 Research Ethics Committee (reference 07/H0308/163).

Maternal Hb in pregnancy and Hb difference - ALSPAC- all antenatal maternal Hb measurements that were taken as part of routine care from venous blood samples were extracted from obstetric records (n = 37,344 measures, a median of 3 per participant, interquartile range: 2-3). We derived early pregnancy and late pregnancy Hb as the mean of all Hb measures taken per participant between 7 and 12 weeks gestation and between 27 and 32 weeks gestation respectively, to align with the blood test schedule in the POPS study. POPS – antenatal maternal venous blood sample Hb measures were taken at booking (~12 weeks) and at ~28 weeks gestation. This resulted in two variables, one for Hb in early pregnancy and one in late pregnancy. Difference in maternal Hb was calculated from the Hb in late pregnancy minus Hb in early pregnancy. Hb was analysed by sulpha haemoglobin measurement using a Sysmex full blood count analyser.

Outcome measures - Birth weight and SGA – In both cohorts, birth weight was abstracted from medical records. LBW was defined as less than 2500 grams. Birth weights were adjusted

for gestational age and fetal sex, and SGA was defined as less than the 10th percentile (13).

PTB – In ALSPAC gestational age was determined by the last menstrual period but adjusted if early pregnancy ultrasound measurements differed by 2 weeks or more, according to the clinical protocol in use at the time. In POPS, gestational age was defined on the basis of ultrasonographic estimation at the time of the first scan. This data was obtained directly from the Ultrasound Department's electronic database (Astraia). PTB was defined as birth before 37 completed weeks of pregnancy.

GDM - In both cohorts this was abstracted from medical records. GDM was diagnosed by standardized criteria used at the time of the pregnancy. In ALSPAC, universal screening of females with a random or fasting blood glucose level, or with an oral glucose tolerance test (OGTT) at 28 weeks gestation was not undertaken, and tests for GDM with an OGTT were offered in participants with established risk factors (family history, previous history of GDM or macrosomic birth, South Asian ethnicity) or persistent glycosuria. In POPS, all participants were offered universal screening at the first antenatal booking visit with random plasma glucose. Participants with random glucose > 7.0 mmol/L (.126 mg/dL) were offered a 75-g OGTT. Participants were screened again at 28 weeks gestation, first with a 50-g glucose challenge test (GCT), followed by a 75-g OGTT if the first GCT was >7.7 mmol/L (.139 mg/dL). Between 2008 and 2011 GDM was diagnosed initially on the WHO criteria. From 2011 onwards, this was replaced with diagnostic criteria adapted from the International Association of Diabetes and Pregnancy Study Groups' recommendations**.**

PET - In both cohorts this data was extracted from medical records and a diagnosis was imposed using the below criteria. In ALSPAC, PET was defined by the International Society

for the Study of Hypertension in Pregnancy (ISSHP) criteria (14). In POPS the ACOG 2013 definition of PET was used (15).

Other variables - In ALSPAC, participants completed up to four postal self-report questionnaires during pregnancy. The questionnaires are available from the study website. Information on socio-demographic factors included data on height, weight, age, self reported race (white/non-white), parity, and cigarette smoking. Data on cigarette smoking were taken from a questionnaire completed at 18 weeks of gestation and defined as; never smoked, temporary smoker, or smoker throughout. BMI was derived from self-reported maternal height and pre-pregnancy weight at a booking appointment.

In POPS, at the 20-week research appointment, participants completed a self-report questionnaire to obtain details about their medical history and socio-demographic characteristics including age, self reported race (white/non-white) and cigarette smoking (defined as for ALSPAC). BMI was calculated using each participants' measured height and their measured weight on the day of their booking scan.

Statistical analysis

In preparing the data biologically implausible values were removed (birth weight < 200g, BMI <10). We tested for departure from linearity in all reported associations using the Box-Tidwell test (16). We used logistic regression models to examine the relationship between Hb and the difference in Hb between early pregnancy and late pregnancy, with pregnancy outcomes: PTB, LBW, SGA, PET and GDM. ORs are reported for a 1g/dl change in Hb. We adjusted for the following confounders: in model 1, maternal age, ethnicity, and in model 2 we also included BMI, parity, smoking status and PET (for PTB, LBW and SGA). In models examining Hb difference between early and late pregnancy (model 3), we included early

pregnancy measurement. Confounders were identified *a priori* from the literature. Analyses included only participants with complete data on all of exposures, outcome measures and potential confounders.

We first analysed ALSPAC and POPs data separately. As only two studies were combined, we pooled the fully adjusted estimates from each cohort in a fixed effect inverse-variance weighted meta-analysis, a weighted average according to their sample size, to give an overall summary estimate (two staged approach) (17). We then tested for heterogeneity/interactions between study and exposures in their relationships with outcomes. There was evidence of heterogeneity for PET and GDM (p-values < 0.05) and therefore for these outcomes, studies were not pooled. Additionally, we analysed the individual participant data from both cohorts in a one-stage approach whilst accounting for clustering within cohort. Furthermore, we estimated associations of anemia defined as <11 g/dl and high Hb defined as ≥13 g/dl compared to normal Hb levels defined as 11 - 12 g/dl with adverse pregnancy outcomes. We used Stata version 15.0 for all analyses.

Results

The participant flow in both the ALSPAC and POPS are shown in **Figure 1.** Complete data on Hb, confounders and outcomes were available for 6,503 and 7,116 ALSPAC pregnancies in early and late pregnancy, respectively, and for 3,790 and 3,018 POPS pregnancies. Socio-demographic characteristics and pregnancy outcomes of ALSPAC and POPS participants included and excluded from analyses with complete data are presented in **Table 1**.

ALSPAC had a higher proportion of participants who smoked throughout pregnancy than POPS. POPS had a higher proportion of overweight participants (BMI ≥ 25 kg/m2), non-white and participants over 35 years than ALSPAC (Table 1). The participants excluded due to not having Hb measures in the specified time points (7-12 weeks or 27-32 weeks gestation

of pregnancy) were comparable to those included in terms of age and BMI. There was a higher proportion of non-white participants in POPS excluded than included (Table 1). There was an increased proportion of those that smoked throughout pregnancy and those participants that had more than two previous pregnancies in ALSPAC of those excluded than included (Table 1).

Mean Hb in ALSPAC was 12.5g/dL (SD= 0.90) and 11.2 g/dL (SD= 0.92) in early and late pregnancy respectively, and 12.7 g/dL (SD=0.82) and 11.4 g/dL (SD= 0.82) in POPS (**Table 2).** Four percent and 2.2 % of participants had a low Hb (Hb <11 g/L) whereas 31.0 % and 36.1% of participants had a high Hb (≥13 g/L) in early pregnancy in ALSPAC and POPS, respectively (Table 2). In late pregnancy 40.4% and 28.8% had a low Hb, whereas 2.6% and 2.7% of participants had a high Hb in ALSPAC and POPS, respectively (Table 2).

Pregnancy outcomes

We found no consistent evidence for departure from linear associations. In pooled one and two stage analyses, higher Hb in early pregnancy was not associated with PTB, LBW and SGA. **(Figure 2, Table 3).** However, higher Hb in late pregnancy was associated with an increased risk of PTB, LBW and SGA **(Figure 3, Table 3).** As there was evidence of heterogeneity in associations of Hb with PET and GDM by study, results for these outcomes were not pooled. Cohort specific associations (adjusted and unadjusted) between maternal Hb level in early and late pregnancy and all pregnancy outcomes are shown in **Table 4.** Higher Hb in early and late pregnancy was associated with a greater risk of PET in the ALSPAC cohort, but there was no association with PET in the POPs cohort (Table 4). In the ALSPAC cohort, there was evidence of an association of higher Hb with GDM in early and late pregnancy, but no association in the POPS cohort at either stage of pregnancy, where the point estimates were in the opposite direction (Table 4). A greater difference in Hb between

early and late pregnancy was inversely associated with greater risk of PTB, LBW, SGA, and PET in both cohorts, but not with GDM (Table 3, Table 4). Categorical analyses showed associations of high Hb with PTB, LBW and SGA in late pregnancy but only PTB in early pregnancy **(Supplementary Table 1).** Categorical analyses showed that low Hb was not associated with adverse pregnancy outcomes in early pregnancy, but was associated with a lower risk of PTB, LBW and SGA in late pregnancy **(Supplementary Table 2).**

Discussion

Main Findings

Our study suggests that higher maternal Hb levels in late pregnancy are associated with PTB, LBW, SGA and PET. It also suggests that a greater difference in Hb between early and late pregnancy and low Hb in late pregnancy are associated with a reduced risk of these outcomes.

Strengths and Limitations

A main strength of our study is the inclusion of two large, contemporary pregnancy cohorts from a high income setting, both with repeat measures of Hb, which allowed us to examine trimester specific associations in our analysis. One limitation of the study is the inclusion of predominantly white participants, and a higher proportion of non-white participants in POPS included than excluded, which limits generalizability. We also did not have data on haemotocrit levels, iron markers of inflammation or iron supplementation details alongside the Hb levels. For GDM the screening and diagnosis methods differed between the cohorts and the sample sizes included in the model in ALSPAC were low (≤60) therefore this outcome has less power making the results difficult to interpret.

Interpretation

In agreement with our findings, a large observational study of 153602 pregnancies in 1988-91 in the UK demonstrated that a failure of Hb to fall below 105g/l (lowest recorded Hb) was associated with an increased risk of LBW and PTB across all ethnic groups (18). They did not however assess trimester specific associations (18). A 2013 systematic review and meta-analysis of 17 observational studies found a U- shaped association curve and that females at both ends of the Hb distribution (low or high) are at increased risk of PTB (4). A further review in 2017 summarized current evidence regarding the association between birth outcomes and maternal Hb concentrations or iron status. Overall, they also confirmed a U-shaped association curve for the risk of adverse pregnancy outcomes with maternal Hb concentrations (9). They found little evidence for the associations between maternal iron status and adverse pregnancy outcomes (9). Two more recent systematic reviews have been undertaken. The first, which included 117 studies, with >80% from LMIC countries, demonstrated increasing risks of adverse maternal and fetal pregnancy outcomes with decreasing pre-conceptual or antenatal Hb levels (10). However, when studies were pooled by continent, a U-shaped distribution was demonstrated for the association between Hb levels, and adverse outcomes in HIC only (n HIC studies=11) (10). The second systematic review, included 97 studies with up to 60% in LMIC. The data demonstrated a U-shaped association curve between Hb and adverse maternal outcomes but a linear association was found between lower Hb and fetal outcomes (LBW, PTB, SGA, perinatal mortality) (11).

A 2015 review into RCTs of daily iron supplementation during pregnancy concluded that iron supplementation reduces the risk of maternal anaemia and iron deficiency in pregnancy, but the positive effect on other maternal and infant outcomes was not clear (19). They concluded that if a woman's initial iron status is low, additional iron is probably beneficial, but if she is iron replete, it may be harmful (19,20). These data suggest that routine iron supplementation in

iron-replete females does not translate into improvements in perinatal outcome, but rather appears to be associated with significantly more adverse pregnancy events (19,20).

The mechanisms underlying the associations between maternal Hb levels and pregnancy outcomes are unclear. Low Hb is thought to be associated with adverse pregnancy outcomes due to iron deficiency, as iron is essential in transporting Hb bound oxygen and to the activity of essential enzymes (21). Iron deficiency-induced changes in maternal metabolism may affect placental structure and functions, nutrient interactions, and fetal organ development (21). Studies in rats suggest that anemia during the fetal period resulted in smaller offspring, with smaller kidneys, and an enlarged heart, all associated with hypertension later in life (21). However, this has not been confirmed in human epidemiological studies (22). Low Hb may also cause chronic hypoxia, inducing a stress response with increased placental corticotropin-releasing hormone (CRH) production, if this occurs too early, it may induce PTB (23). Interestingly, one study hypothesized that the effect of low maternal Hb levels on stillbirth could be mediated via low fetal birth weight or infections, however the data did not support these models suggesting that the effect of Hb levels on stillbirth is independent of these factors (24).

It has been hypothesized that high Hb concentrations increase blood viscosity, with or without change in the plasma volume and reduce placental perfusion, leading possibly to placental and fetal hypoxia (25). In normal pregnancy Hb levels fall in the third trimester due to plasma expansion and a mid-trimester fall of Hb concentration may therefore, be optimal, as it reflects good expansion of plasma volume. These mechanisms suggest a causal effect of Hb on adverse placental conditions in pregnancy. However, it is also possible that adverse placental conditions cause a lack of hemodilution which impacts on the Hb levels in pregnancy and confounds any potential relationship (26). Another hypothesis is that excess

iron intake may contribute to oxidative stress via increases in circulating non–transferrin bound iron, leading to lipid peroxidation and DNA damage of placental cells, or adversely impacting the systemic response to inflammation and infection (27-29). One hypothesis has postulated that a surge of oxygen and iron can results in excessive membrane lipid peroxidation and ferroptosis at the maternal-fetal interface, primarily in trophoblast cells, leading to shallow endovascular invasion of extravillous cytotrophoblast and suboptimal remodeling of the maternal spiral arteries, the pathologic hallmarks of pre-eclampsia and disorders of placental dysfunction (30).

Clinical Implications - The non-linear relationship between Hb and outcome in other studies may reflect differences in the determinants of Hb concentration in different populations. In low-income populations, low Hb may reflect dietary inadequacy in iron, with direct adverse effects on the pregnancy due to inadequate Hb synthesis. It may also be a marker for other associations, such as generally poor quality or inadequate diet or parasitic disease. However, the populations studied in the present analysis were high income and the participants had generally low levels of deprivation. In these populations, the main determinant of low Hb is hemodilution, which is a physiological feature of normal pregnancy. Hence, a high Hb may represent inadequate haemodilution. This aligns with our findings that a larger Hb change during pregnancy was associated with a lower risk of adverse pregnancy outcomes. We conclude that in a high-income setting, Hb levels principally reflect physiological adaptation to pregnancy and this could explain the positive linear associations observed between Hb and pregnancy complications, which are in opposition with many of studies in different pregnancy cohorts. As anaemia has multiple causes, investigations to better explore these could understand further the discrepancies in the current evidence.

Research Implications - Addressing anemia to improve pregnancy outcomes is a vital public health issue in populations suffering under nutrition. However, higher levels of Hb may also identify females who might be at increased risk of adverse pregnancy outcomes in high-income settings, regardless of whether associations between Hb and outcomes are causal or not. Further future research should also explore the definition of normal or 'healthy' ranges of Hb and iron levels in different trimesters and in different populations to guide management and screening in pregnancy.

Conclusions

Our findings suggest that higher Hb later in pregnancy is associated with PTB, LBW, SGA and PET and more strongly than first trimester Hb concentrations. This may reflect a pathological process and impaired hemodilution, and therefore may be used to identify females at risk of these conditions in high-income settings.

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Authorship

All authors meet the ICMJE criteria for authorship

CB – conception, methodology, data analysis, data interpretation original draft writing

GC - methodology, data analysis, data interpretation, reviewing and editing.

US – methodology, draft reviewing and editing.

GS - methodology, draft reviewing and editing.

AF – conceptualisation, methodology, data analysis, data interpretation, draft reviewing and editing. All authors (CB, GC, US, GS, AF) have read and approved the final manuscript.

Ethics approval

Procedures followed were in accordance with the ethical standards of the institution or regional committee on human experimentation and that approval was obtained from the relevant committee on human subjects. Ethical approval for the study was obtained from the Cambridgeshire 2 Research Ethics Committee (reference 07/H0308/163). Ethics approval was granted by the ALSPAC Law and Ethics committee and the local research ethics committee, in compliance with the Declaration of Helsinki http://www.bristol.ac.uk/alspac/researchers/research-ethics/.

Data Sharing Plan

Data described in the manuscript, code book, and analytic code will be made available upon request pending [e.g., application and approval, payment, other].

Analysis scripts can be found on the following GitHub page: https://github.com/gc13313/Hbinpreg.

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Table 1. Characteristics (n (%)) and outcomes of ALSPAC and POPS participants with hemoglobin (Hb) measures in early pregnancy (7 to 12 weeks gestation) (n= 6,505 and n= 3,790) and late pregnancy (27 to 32 weeks gestation) (n=7,121 and n= 3,018) by inclusion status

		ALSPAC n (%)			**ALSPAC n (%)**			**POPS n (%)**			**POPS n (%)**		
		Early pregnancy 7 - 12 weeks gestation			**Late pregnancy 27 - 32 weeks gestation**			**Early pregnancy 7 - 12 weeks gestation**			**Late pregnancy 27 -32 weeks gestation**		
		Included n= 6,503	**Excluded n= 3,316**	**p value**	**Included n= 7,116**	**Excluded n= 2,703**	**p value**	**Included n=3,790**	**Excluded n= 30**	**p value**	**Included n= 3,018**	**Excluded n= 1,080**	**p value**
Age	**≤35**	5,856 (90.1)	2,921 (88.1)		6,377 (89.6)	2,400 (84.8)		3,210 (84.7)	264 (85.7)		2,549 (84.5)	925 (85.6)	
	> 35	647 (9.9)	395 (11.9)	<0.01	739 (10.4)	303 (11.2)	0.24	580 (15.3)	44 (14.3)	0.63	469 (15.5)	155 (14.4)	0.35
Age - Mean (SD)		28.5 (4.6))	28.4 (5.1)		28.5 (4.7)	28.3 (4.9)	0.23	29.9 (5.2)	29.8 (4.9)	0.70	30 (5.1)	29.6 (5.2)	0.02
Race	**White**	6,376 (98.0)	3,232 (97.5)		6,960 (97.8)	2,648 (98.0)		3,582 (94.5)	282 (96.6)		2,860 (94.8)	1,004 (93.0)	
	Non-white	127 (2.0)	84 (2.5)	0.06	156 (2.2)	55 (2.0)	0.63	208 (5.5)	26 (8.4)	0.03	158 (5.2)	76 (7.0)	0.03
BMI (kg/m2)	≤18.5	290 (4.5)	167 (5.0)		328 (4.6)	129 (4.8)		59 (1.6)	4 (1.3)		42 (1.4)	21 (2.0)	

	>18.5 - 24.9	4,858 (74.7)	2,465 (74.4)		5,318 (74.8)	2,005 (74.2)		2,141 (58.2)	186 (61.6)		1,687 (57.4)	640 (61.5)	
	≥25.0 - 29.9	1,006 (15.5)	489 (14.7)		1,072 (15.1)	423 (15.7)		1,064 (28.9)	80 (26.5)		867 (29.5)	277 (26.5)	
	≥30 .0- 39.9	322 (5.0)	177 (5.3)		361 (5.1)	138 (5.1)		367 (10.0)	27 (8.9)		305 (10.4)	89 (8.5)	
	≥40.0	24 (0.4)	17 (0.5)		34 (0.5)	7 (0.3)	0.58	48 (1.3)	5 (1.7)	0.77	39 (1.3)	14 (1.3)	0.07
BMI- Mean (SD)		23.0 (3.8)	23.0 (3.9)	0.39	23.0 (3.8)	23.0 (3.8)	0.99	25.1 (4.7)	24.9 (4.8)	0.49	25.2 (4.7)	25.0 (4.8)	0.27
Smoking in pregnancy	Never	5,071 (78.0)	2,467 (74.4)		5,546 (77.9)	1,992 (73.7)		2,229 (58.8)	202 (65.6)		1,794 (59.4)	637 (59.0)	
	Temporary	407 (6.3)	231 (7.0)		468 (6.6)	170 (6.3)		1,062 (28.0)	74 (24.0)		831 (27.5)	305 (28.2)	
	Throughout	1,025 (15.8)	618 (18.7)	<0.01	1,0102 (15.5)	541 (20.0)	<0.01	499 (13.2)	32 (10.4)	0.06	393 (13.0)	138 (12.8)	0.09
Parity	≤ 2	5,353 (82.3)	2,616 (78.9)		5,480 (82.1)	2,219 (78.8)		3,970 (100.0)	308 (0.0)		3.018 (100.0)	1,080 (100.00)	

	>2	1,150 (17.7)	700 (21.1)	<0.01	1,276 (17.9)	574 (21.1)	<0.01	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Preterm birth	Yes	273 (4.2)	170 (5.1)		307 (4.3)	136 (5.0)		164 (4.4)	13 (4.2)		139 (4.6)	40 (3.7)	
	No	6,230 (95.8)	2,146 (94.9)	0.04	6,809 (95.7)	2,567 (95.0)	0.13	3,624 (95.6)	295 (6.1)	0.90	2,892 (95.8)	1,040 996.3)	
Low birth weight	Yes	227 (3.5)	125 (3.8)		239 (3.4)	113 (4.2)		158 (4.2)	12 (3.9)		126 (4.2)	44 (4.1)	
	No	6,276 (96.5)	3191 (96.2)		6,877 (96.6)	2,590 (95.8)		3,632 (95.8)	296 (96.1)		2,892 (95.8)	1,036 (95.9)	
Pre-eclampsia	Yes	146 (2.3)	72 (2.2)		169 (2.4)	49 (1.8)		256 (6.8)	15 (4.9)		196 (6.5)	75 (6.9)	
	No	6,357 (97.7)	3,233 (97.8)		6,947 (97.6)	2,654 (98.2)		3,534 (93.2)	293 (95.1)		2,822 (93.5)	1,005 (93.1)	
Gestational diabetes mellitus	Yes	47 (0.7)	72 (2.2)		58 (0.8)	16 (0.6)		171 (4.5)	13 (4.2)		149 (4.9)	35 (3.2)	
	No	6,456 (99.3)	3,244 (97.8)		7,058 (99.2)	2,687 (99.4)	0.25	3,619 (95.5)	295 (95.8)	0.81	2,869 (95.1)	1,045 (96.8)	0.02

Small for gestational age	Yes	457 (7.0)	27 (0.8)		525 (7.4)	168 (6.2)		340 (9.0)	25 (8.1)		260 (8.6)	105 (9.7)	
	No	6,046 (93.0)	3,289 (99.2)		6591 (92.6)	2,535 (93.8)	0.04	3,450 (91.0)	283 (91.9)	0.61	2,758 (91.4)	975 (90.3)	0.27

ALSPAC: The Avon Longitudinal Study of Parents and Children study, GDM – gestational diabetes mellitus, Hb – haemoglobin, LBW -Low birthweight < 2500 grams, PET – preeclampsia , POPS: Pregnancy Outcome Prediction Study SGA- small for gestational age < 10^{th} birth centile, p values reported are based on the analysis of variance test for means Kruskal Wallis test for medians and chi squared for proportions

Table 2 - Hemoglobin (Hb) in the ALSPAC and POPS cohorts

	ALSPAC		**POPS**	
Hb gd/L	**Early pregnancy 7 - 12 weeks gestation** **n= 6,503**	**Late pregnancy 27 - 32 weeks gestation** **n= 7,116**	**Early pregnancy 7 - 12 weeks gestation** **n= 3,790**	**Late pregnancy 27 - 32 weeks gestation** **n= 3,018**
Mean (SD)	12.5 (0.90)	11.2 (0.92)	12.7 (0.82)	11.4 (0.82)
Median (range)	12.5 (7.8-15.9)	11.2 (8.0-15.6)	12.7 (9.0-16.1)	11.4 (7.8-15.1)
<11 (%)	269 (4.1)	2,874 (40.4)	84 (2.2)	868 (28.8)
11-12 (%)	4,209 (64.7)	4,064 (57.1)	2,339 (61.7)	2,070 (68.6)
≥13 (%)	2,027 (31.2)	183 (2.6)	1,367 (36.1)	80 (2.7)

ALSPAC: The Avon Longitudinal Study of Parents and Children study, Hb – haemoglobin, POPS: Pregnancy Outcome Prediction Study

Table 3 – Pooled estimates (one stage approach) of associations of hemoglobin - Hb (1g/dL) with pregnancy outcomes (odds ratio, 95%CI), early pregnancy (7-12 weeks gestation) n= 10,293, late pregnancy (27-32 weeks gestation) n= 10.134 and difference in Hb between trimesters n = 7,768

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Outcome measure	**Early pregnancy (7-12 weeks gestation**		**Late pregnancy (27–32 weeks gestation)**		**Difference in Hb between trimesters**	
	Model 1	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 3**
	n= 10,293		**n=10,134**		**n= 7,768**	
Preterm birth	1.1 (1.03, 1.18)	1.09 (0.99, 1.20)	1.5 (1.36, 1.65)	1.46 (1.37, 1.58)	0.8 (0.78, 0.82)	0.7 (0.67, 0.73)
Low birth weight	1.14 (0.98, 1.32)	1.11 (0.92, 1.34)	1.78 (1.40, 2.27)	1.77 (1.38, 2.28)	0.69 (0.63, 0.75)	0.58 (0.47, 0.70)
Small for gestational age	1.05 (0.94, 1.16)	1.06 (0.94, 1.18)	1.41 (1.24, 1.61)	1.46 (1.28, 1.66)	0.75 (0.68, 0.83)	0.65 (0.54, 0.78)

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Hb – hemoglobin, , LBW – low birth weight, PTB – preterm birth, SGA – small for gestational age, Pooled estimates (one stage approach) of associations of hemoglobin – Hb (1g/dL) with pregnancy outcomes (odds ratio, 95%CI)

Model 1 - adjusted for maternal age and ethnicity.

Model 2 - model 1 + parity, smoking, BMI, PET GDM

Model 3 – model 2 + first trimester Hb

Table 4 - Associations of hemoglobin levels (Hb, g/dL) with pregnancy outcomes (odds ratio, 95%CI), early pregnancy – 7-12 weeks gestation (n= 6,503 ALSPAC, n= 3,790 POPS), late pregnancy -27-32 weeks gestation (n = 7,116 ALSPAC, n= 3,018 POPS), difference in Hb between trimesters (n= 4,895 ALSPAC, n= 2,873 POPS)

Outcome measures	**Early pregnancy 7-12 weeks gestation**		**Late pregnancy 27 – 32 weeks gestation**		**Difference in Hb between early and late pregnancy**	
	Model 1	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 3**
ALSPAC	**n= 6,503**		**n= 7,116**		**n= 4,895**	
Preterm birth	1.13 (0.99, 1.30)	1.13 (0.98, 1.30)	1.55 (1.37, 1.76)	1.49 (1.31, 1.70)	0.8 (0.68, 0.93)	0.7 (0.57, 0.84)
Pre-eclampsia	1.48 (1.22, 1.78)	1.36 (1.12, 1.64)	1.68 (1.42, 1.99)	1.53 (1.29, 1.82)	0.82 (0.67, 1.00)	0.64 (0.51, 0.82)
Low birth weight	1.21 (1.04, 1.41)	1.21 (1.04, 1.41)	1.95 (1.69, 2.26)	1.96 (1.68, 2.27)	0.67 (0.56, 0.80)	0.52 (0.42, 0.65)
Small for gestational age	1.109 (0.98, 1,21)	1.11 (0.99, 1.24)	1.48 (1.34, 1.63)	1.53 (1.38, 1.69)	0.73 (0.64, 0.82)	0.6 (0.52, 0.69)
Gestational diabetes mellitus	1.72 (1.24, 2.39)	1.51 (01.08, 2.11)	1.51 (1.13, 2.01)	1.35 (1.01, 1.79)	1.22 (0.86, 1.73)	0.96 (0.64, 1.43)
POPS	**n= 3,790**		**n= 3,018**		**n= 2,873**	

Preterm birth	1.05 (0.87, 1.27)	1.02 (0.84, 1.24)	1.36 (1.10, 1.68)	1.35 (1.09, 1.66)	0.82 (0.58, 0.97)	0.73 (0.56,0.95)
Pre-eclampsia	1.27 (1.08, 1.48)	1.17 (0.99, 1.37)	1.1 (0.92, 1.32)	1.03 (0.86, 1.23)	1.01 (0.83, 1.23)	0.96 (0.76, 1.20)
Low birth weight	1.02 (0.84, 1.24)	0.99 (0.81, 1.20)	1.44 (1.16, 1.80)	1.44 (1.16,1.79)	0.73 (0.57, 0.93)	0.65 (0.49, 0.86)
Small for gestational age	0.96 (0.83,1.09)	0.98 (0.86, 1.13)	1.24 (1.06, 1.45)	1.29 (1.10, 1.51)	0.82 (0.69,97)	0.75 (0.61,0.91)
Gestational diabetes mellitus	1.09 (0.90, 1.32)	0.98 (0.81, 1.19)	0.89 (0.73, 1.09)	0.83 (0.68, 1.02)	1.2 (0.96, 1.50)	1.26 (0.97, 1.64)

ALSPAC: The Avon Longitudinal Study of Parents and Children study, CI – confidence interval, GDM – gestational diabetes mellitus, Hb – haemoglobin, LBW -Low birthweight < 2500 grams, OR – odds ratio, PET – pre-eclampsia , POPS: Pregnancy Outcome Prediction Study SGA- small for gestational age < 10^{th} birth centile, Cohort specific associations of hemoglobin levels- Hb g/dL with pregnancy outcomes (OR, 95%CI)

Model 1 – adjusted for maternal age and ethnicity.

Model 2 – model 1 + parity, smoking, BMI, PET, GDM

Model 3 – model 2 + first trimester Hb

Figure 1: Flowchart of participants in ALSPAC and POPS

ALSPAC - The Avon Longitudinal Study of Parents and Children study, Hb – hemoglobin POPS -Pregnancy Outcome Prediction Study

Figure 2: Pooled estimates (two-stage approach) of associations of early pregnancy (7- 12 weeks gestation) hemoglobin – Hb (1g/dL) with pregnancy outcomes (odds ratio, 95%CI), n= 10,293 (ALSPAC n = 6503 POPS n = 3790)

Hb – hemoglobin

Figure 3: Pooled estimates (two-stage approach) of associations of late pregnancy (27 – 32 weeks gestation) hemoglobin with pregnancy outcomes (odds ratio, 95%CI) n= 10,134 (ALSPAC n = 7116 POPS n = 3018)

Hb – hemoglobin